ABSTRACT
Batch sorption equilibrium experiments using free and immobilized Penicillium cyclopium biomass in PVA-hydrogel were carried out using ternary mixture of corresponding metal ion solutions. The metal binding ability of the hybrid hydrogel for Cu(II), Co(II) and Fe(III) ions was determined by atomic absorption spectrophotometer. The performance of free and immobilized biosorbent was evaluated by sorption kinetics and sorption capacities for different metal ions in the mixture. The immobilized system showed higher removal efficiency (expressed as mg metal ions removed per mg metal ions added) in comparison to the free-cell system. In addition, the immobilized system showed two-fold shorter time for reaching the equilibrium in comparison to the free cells. The pseudo-second order kinetic model was applied to the multi-component experimental data and important kinetic parameters of the biosorption process were calculated. The data obtained are in agreement with the assumption that the external mass transfer limitation in the immobilized system can be neglected and biosorption is chemisorption controlled. The immobilized system of organic polymer with entrapped fungal biomass showed high biosorption performance and may be used successfully for the removal of Cu(II), Co(II) and Fe(III) ions from ternary mixed solutions.
Academy of Sciences was used in this study. The strain was routinely maintained on potato/glucose agar slant and stored at 4 °C. Subcultures were made every 3 months.

The medium for the development of the inoculum was (g/l): glucose 30, corn steep liqueur 40, peptone 10, MgSO₄·7H₂O 0.5, KH₂PO₄ 10, pH 4.8 - 5. The fermentation medium comprised (g/l): glucose 30, corn steep liqueur 45, pH 4.8 - 5. The inoculum was obtained by introducing 0.1 ml spore suspension (about 10⁶ spores per ml) in Erlenmeyer flasks containing 75 ml sterile medium. The flasks were cultivated at 30 °C on a rotary shaker at 220 rpm for 18 h. The fermentation process took place under the same conditions with 10% (V/V) inoculum being introduced into the sterile liquid medium.

Biomass at the 72nd hour of cultivation was separated by filtration, washed with deionized distilled water, and preweighed amount of wet biomass was used as a biosorbent.

Synthesis of hydrogel and biomass immobilization
Poly(vinyl alcohol) (Fluka) and glutaraldehyde (50% aqueous solution) (Fluka) were used as received.

Aqueous solution of PVA of concentration 10 wt.% was used for hydrogel preparation. Hydrogels were obtained by precipitation of the PVA from its aqueous solution into absolute ethanol, polymer being simultaneously crosslinked with glutaraldehyde using HCl as catalyst. Vigorous stirring with electromagnetic stirrer was applied during the first stage of the hydrogel formation. The mixture was stirred at room temperature for 10 min and then the reaction was let to proceed at room temperature for at least 6 hours. The hydrogel obtained was washed with deionized water until neutral pH.

Immobilization of *P. cyclopium* was performed by dispersing a preweighed amount of wet biomass (Table 1) in the PVA aqueous solution prior to the precipitation. The reaction conditions were the same as those applied to the pure PVA hydrogel synthesis.

Swelling degrees of the hydrogels were measured gravimetrically. The swelling degree \( W \) was defined as:

\[
W = \frac{w - w_o}{w_o} \times 100
\]

where \( w_o \) and \( w \) are the weight of the dry and swollen sample respectively.

**Scanning electron microscopy**
The scanning electron microscopy was done on a JEOL JSM-5510. Prior to investigation the hydrogel samples were fractured in liquid nitrogen, freeze-dried and finally gold coated. The samples’ cross-sections were examined.

**Biosorption equilibrium**
Classically, biosorption experiments were carried out in batches in 500 ml Erlenmeyer flasks, as follows: preweighed biosorbent samples (wet or immobilized biomass) with concentration varying from 1 to 2 g/l (dry weight) were examined. Each sample was added to 100 ml ternary solution containing 20 mg/l of every heavy metal ion (Cu²⁺, Co²⁺ and Fe³⁺) prepared by dissolving corresponding sulfate salts in bidistilled water. Biosorption studies were carried out by varying the initial pH of the solution from 3.5 to 6.5 using 0.1N NaOH. The mixtures were then agitated at 220 rpm on a rotary shaker for 30 min at 30 °C necessary to reach the sorption equilibrium. Then the content of the flasks was separated by filtration using a Whatman №1 filter paper.

**Kinetic studies**
Biosorption kinetics were investigated using biomass loaded PVA-hydrogel (1.5 g/l) and freely suspended biomass at the same concentration. Samples were taken periodically for analysis.

In order to investigate the mechanism of biosorption and the rate controlling step such as mass transport and chemical reaction processes, the pseudo-second order kinetic model
was used to test experimental data in a system using PVA-immobilized biomass as a biosorbent. If the sorption process follows a second order mechanism, the pseudo-second order chemisorption kinetic rate equation for each component in the mixture is expressed as (7, 18):

\[
\frac{t}{q} = \frac{1}{k_2 q_e} + \frac{t}{q_e}
\]

where \( k_2 \) can be regarded as the initial sorption rate as \( t \) approaches 0, \( k_2 \) is the second order rate constant and \( q_e \) is the equilibrium uptake value. If the pseudo-second order kinetics is applicable, the plot of \( t/q \) versus \( t \) gives a linear relationship, from which \( q_e \) and \( k_2 \) can be determined from the slope and intercept of the plot (11).

**Analytical determinations**

The concentration of the metal ions in the filtrates was determined using atomic absorption spectrophotometer with an air/acetylene flame (model 2380; Perkin Elmer, Uberlingen, Germany).

Uptake of metal ions was calculated from a metal mass balance yielding (22):

\[
q = \frac{V(C_i - C_f)}{m}
\]

where: \( q \) is mg metal ions per g dry biosorbent, \( V \) is the reaction volume (l), \( C_i \) and \( C_f \) are the initial and residual metal concentrations (mg/l), respectively, and \( m \) is the amount of dry biosorbent (g).

Aliquots of wet biomass as well as of immobilized biomass, followed by drying for 48 h at 85 °C, were considered as dry biosorbent to calculate the uptake.

The efficiency of heavy metal removal was calculated from the amount of metal ions adsorbed on the biosorbent and the amount of metal ions added in the medium, by the following equation:

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**Fig. 1.** Scanning electron micrographs of PVA-hydrogel particles (a) and PVA-hydrogel particles loaded with *Penicillium cyclopium* biomass (b): original magnification ×2.500, bar = 10 µm.

**Fig. 2.** Effect of biosorbent concentration on the specific heavy metal uptake by free cells (a) and PVA-immobilized cells (b). Heavy metal concentrations (mg/l): Cu(II) – 20.5, Co(II) – 19.8, Fe(III) – 20.9; pH 4.0; agitation – 220 rpm; contact time – 30 min. Average values of three independent experiments are given.
Reproducibility
Unless indicated, the data shown are average from three separate experiments. Variations up to 10% were observed.

Results and Discussion
Characterization of the PVA-matrix
Scanning electron microscopy (SEM) analysis revealed that the structure of the blank PVA-hydrogel (Fig. 1a) was intensive undulate with numerous folds and pores allowing inclusion of the fungal biomass into the unoccupied areas. The structural network of the polymeric hydrogel has extensive surface area with a lot of cavities, which makes it an ideal matrix for immobilization of microbial cells. This structure was distinct from that reported earlier for PVA beads formed by using other methods, where there were not as many folds and cavities found (6, 12). PVA-hydrogel particles loaded with fungal biomass showed a dense distribution of filamentous biomass tightly packed within the unoccupied cavities of the PVA-hydrogel matrix (Fig. 1b).

Effect of biosorbent concentration on the equilibrium uptake values
The effect of variation of biosorbent dosage on the removal of heavy metals from mixed aqueous solution is shown in Fig. 2. It was found that Co(II) ion uptake is independent of the biosorbent concentration but is highly influenced by the immobilization. PVA-entrapped biomass showed about 4-fold increase in the Co(II) uptake compared to the freely suspended cells. In the case of Cu(II) and Fe(III) the equilibrium concentration of each heavy metal in the solution increased with increasing of the doses of biosorbent up to 1.5 g/l and then dropped down. It is evident from the data presented on Fig. 2 that for the given initial concentration of the heavy metals, the optimal biosorbent dosage was found to be about 1.5 g/l for both free and immobilized biomass. Thus, when 1.5 g/l or immobilized biomasses were used as biosorbents, the maximal values of total sorption capacity for the tertiary mixture of heavy metals were reached. In contrast, a reverse trend was found at higher biosorbent dosage of 2 g/l. This decrease in the heavy metal uptake was attributed to electrostatic interactions between the reactive groups of the binding sites which take part in biosorption (17, 19). Such behavior has previously been observed at varying dosage of PVA-immobilized yeast cells (19), where an increase in biosorbent concentration led to a decrease in the specific metal uptake. The possible explanations of such behavior include limited availability of the solute, electrostatic interactions, interference between binding sites, etc. The decrease in the biosorbent concentration in the suspension at a given metal concentration increases the metal/biosorbent ratio, and thus an increase in the specific metal uptake could be expected as long as the biosorbent is not saturated.

Effect of initial pH on the equilibrium uptake values
Immobiled biosorbent of optimal concentration of 1.5 g/l was placed in contact for 30 min with a mixed solution of Cu(II), Co(II) and Fe(III) ions at equal concentrations of approximately 20 mg/l. Initial pH values of the solutions were varied from 3.5 to 6.5. Fig. 3 shows the effect of the initial pH on the heavy metal biosorption in mixed solution. At initial pH of 3.5 there was little biosorption of Cu(II) and Co(II) ions. At pH 2.5 the biosorption of metals was negligible, except for Fe(III) (data not shown). The biosorption of Cu(II) and Co(II) ions began at the initial pH of 3.5. A sharp increase in biosorption capacity as well as in removal efficiency of the system was observed in the pH range from 4.5 to 6.5. The residual concentration of Fe(III) in the solution, however, tended to be negligible for the entire pH interval and maximal removal efficiency of the system was reached. It has been reported that different metals have different pH optima due to the different solution chemistry of the metals (5, 16). The low biosorption capacity at pH below 3.5 was attributed to hydrogen ions that compete with metal ions on the sorption sites (19).

Kinetic studies on the biosorption process
The batch kinetic studies in which wet biomass was used (FC – 1.5 g/l as dry weight) showed that at an initial pH of 4.0 (chosen to prevent the precipitation of Fe(III) ions), the equilibrium time for each of the heavy metals tested was about 30 minutes (Fig. 4). Kinetic studies using PVA-immobilized biomass were also conducted to investigate whether the equilibrium time would be changed when the biomass used was in the form of immobilized cells. It can be seen from Fig. 4 that, similar to the case of biosorption using free cells, the biosorption by immobilized biomass also consisted of two phases: a primary rapid phase and a secondary slow phase. The first phase was about 15 - 20 min, which is longer than the 3 - 5 min observed in the case of immobilized biosorbent. As it has been reported earlier (10, 20), the functional groups on the biosorbent may be modified after immobilization, and hence the role of the HO-groups in the process of metal ions biosorption may

\[
\% \text{ removal} = \frac{\text{mg heavy metal ions removed}}{\text{mg heavy metal ions added}} \times 100
\]
be increased. Thus, PVA-immobilized biomass had higher surface area than free cells and shorter time was needed for the first phase of the process. In this case the equilibrium time was twice as shorter as that obtained for the free-cell system. This shorter equilibrium time might be attributed to modification of functional groups on biosorbent after immobilization.

The pseudo-second order reaction model applied by Kargi and Cikla (11) was used to analyze the adsorption kinetics. Fig. 5 illustrated the model plots $t/q$ vs. $t$ in the case of IC only. The pseudo-second order reaction rate model adequately described the biosorption kinetics in mixed solutions using IC as a biosorbent, as demonstrated by Tsekova et al. (21) in batch kinetic studies using FC.

The parameter values of $k_s$, $h$ and $q_e$ for Cu(II), Co(II) and Fe(III) biosorption are presented in Table 2. The values of $q_e$ calculated with the model used ($q_e$ calc.) were 7.8, 8.5 and 13.4 mg/g for Cu, Co and Fe, respectively and these values were found to be close to the ones obtained experimentally ($q_e$ exp.). The correlation coefficients ($R^2$) obtained for the second-order kinetic model were close to 1 for all ions in the mixture.

These data suggest the applicability of the second order kinetic model based on the assumption that the rate limiting step may be chemisorption involving valence forces through sharing or exchange of electrons between sorbent and sorbate.

**Conclusions**

*Penicillium cyclopium* biomass immobilized into PVA-hydrogel particles using glutaraldehyde as a crosslinking agent proved to be successful for removing of heavy metals from mixed solutions. The presented immobilized system showed higher removal efficiency in comparison to the free-cell system. The uptake capacity of this system toward heavy metals removing is higher to that reported elsewhere in the literature. In addition, the process of the PVA-hydrogel preparing is relatively easy and the biomass load that can be incorporated makes it an attractive method for different types of biomass, including waste products.

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